

# The Emerging Role of Natural Killer Cells in Innate and Adaptive Immunity

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## ABSTRACT

In the early host defense system, effector function of natural killer (NK) cells results in natural killing against target cells such as microbe-infected, malignant, and certain allogenic cells without prior stimulation. NK cell cytotoxicity is selectively regulated by homeostatic prevalence between a repertoire of both activating and inhibitory receptors, and the discrimination of untransformed cells is achieved by recognition of major histocompatibility complex (MHC) class I alleles through inhibitory signals. Although it is well known that the bipotential T/NK progenitors are derived from the common precursor, functional mechanisms in terms of the development of NK cells remain to be further investigated. NK cells are mainly involved in innate immunity, but recent studies have been reported that they also play a critical role in adaptive immune responses through interaction with dendritic cells (DC). This interaction will provide effector functions and development of NK cells, and elucidation of its precise mechanism may lead to therapeutic strategies for effective treatment of several immune diseases. (**Immune Network 2004;4(4):205-215**)

**Key Words:** Natural killer cells, innate immunity, adaptive immunity, development, function, NK receptors, tumor, major histocompatibility complex, dendritic cells

## Introduction

Natural killer (NK) cells play a central role in early host defense via interaction of activating receptors with their ligands on virus-infected cells, tumor cells, and some normal allogenic cells in the absence of prior sensitization (1). NK cells have an ability to discriminate between normal cells and cells lacking the expression of MHC class I molecules due to the recognition through NK inhibitory receptors that specific for major histocompatibility complex (MHC) class I molecules. Their morphological characteristic showed a large granular lymphocyte (LGL)-like morphology based on the presence of densely staining azurophilic granules in their cytoplasm. In general, phenotype of NK cells is characterized by the expression of the

CD56 and CD16 (in human), NKR-P1C (NK1.1 in mouse, CD161 in human), DX5, Ly49 (in mouse; these are restricted to certain mouse strains) surface antigen and the lack of CD3. They comprise approximately 10 to 20% in normal peripheral blood lymphocytes, 15 to 25% in liver lymphocytes, and 1 to 5% in spleen lymphocytes. The majority (comprising 90% of total NK cells) of human NK cells have low-density expression of CD56 (CD56<sup>dim</sup>, more cytotoxic) and express high levels of Fcγreceptor III (FcγRIII, CD16), whereas ~10% of NK cells are CD56<sup>bright</sup>CD16<sup>dim</sup> or CD56<sup>bright</sup>CD16<sup>-</sup> (2,3). Whereas effector function of NK cells can be stimulated by cytokines including interleukin-2 (IL-2), IL-12, IL-15, IL-18, IL-21, type I interferon (IFNαβ) in combination with differential engagement of cell surface receptors, they produce immunoregulatory cytokines such as IFN-γ, IL-5, IL-10, IL-13, tumor necrosis factor (TNFα), and granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as a number of

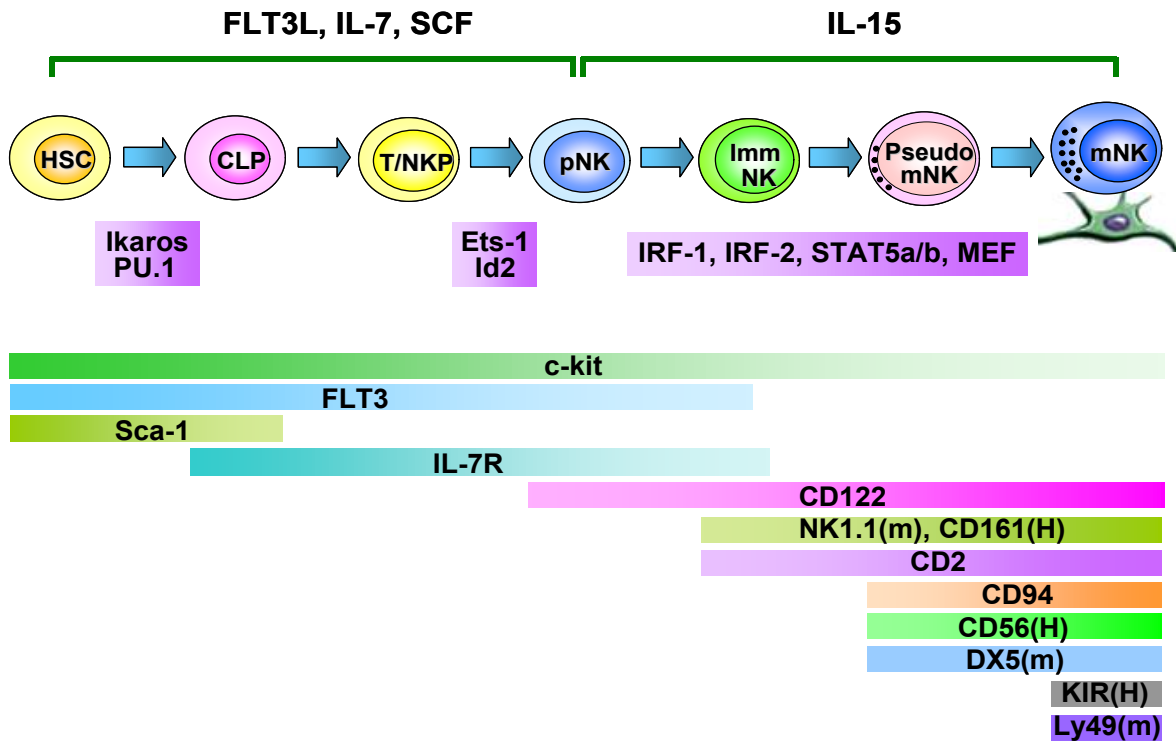
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chemokines following interaction of NK receptors with their ligands (4) NK cells are also able to induce spontaneous cytolytic activity independent on MHC class I antigen-specific recognition to kill target cells ('missing self' hypothesis) contrary to cytotoxic T lymphocytes (CTL) (5). This means that signaling through inhibitory NK receptor can protect from NK cell-mediated cytotoxicity in healthy cells expressing normal levels of MHC class I molecules. Therefore, down-regulated MHC class I expression, which can be occurred by malignant transformation or virus infection of target cells may give rise to NK cell-mediated killing (6). They also mediate antibody-dependent cellular cytotoxicity (ADCC) through FcγRIII (CD16), a receptor molecule that specifically binds the Fc portion of an antibody (7). In addition to functional roles of NK cells in the early defense to infection, NK cells are capable of the regulation of adaptive immune responses by inducing T cell-mediated memory (8), and B cell-mediated autoimmunity (9).

**NK cell development by cytokines and transcription factors**

Although NK cells have been well known to be derived from pluripotent hematopoietic stem cells (HSCs) (10,11), the ontogeny of NK cells has not been fully understood yet. HSCs (Lin<sup>-</sup>CD34<sup>+</sup> in human, Lin<sup>-</sup>c-kit<sup>+</sup>Sca2<sup>+</sup> in mouse) from fetal thymus, fetal liver, umbilical cord blood, and adult bone marrow (BM) have a potential to develop into the common T/NK bipotent progenitors (11,12), which are committed to NK cell precursors (pNKs) upon *in vitro* culture in combination with IL-7, stem cell factor (SCF), and Fms-like tyrosine kinase 3 ligand (flt3L) (13)(Fig. 1). Even though pNK cells are widely distributed in the BM, fetal thymus, blood, spleen, and liver, they are absent of both cytolytic activity as well as ability to produce large amounts of IFN-γ, but express CD122 (IL-2/15Rβ<sup>+</sup>). In addition, low levels of NKp46, NKp30 and 2B4 are also expressed in pNK cells before the expression of CD94/NKG2A and KIRs, and their appearance correlated with the



**Figure 1.** *In vitro* model of NK cell development from hematopoietic stem cells (HSCs). Bipotent T/NK progenitors (T/NKP) derived from HSCs (c-kit<sup>+</sup>Sca-1<sup>+</sup>) have originated in common lymphoid progenitors (CLP) upon *in vitro* culture in combination with FLT3L, IL-7, and SCF. From T/NKP, further cultures of NK cell precursors (pNK; CD122<sup>+</sup>) with IL-15 alone arise to immature NK cells (CD2<sup>+</sup> and CD161<sup>+</sup> in human, NK1.1<sup>+</sup> in mouse), which can differentiate sequentially to pseudomature cytolytic NK cells (CD94<sup>+</sup>, CD56<sup>+</sup>CD161<sup>+</sup>KIR<sup>-</sup> in human and NK1.1<sup>+</sup>DX5<sup>+</sup>Ly49<sup>-</sup> in mouse). The interaction of pNK cells with stromal cells can generate mature NK cells (KIR<sup>+</sup> in human, and Ly49<sup>+</sup> in mouse) with cytolytic activity. At differential stages of cells during NK cell development, the expression of transcription factors and developmental cell surface markers is highlighted in color.

acquisition of cytolytic activity (14). When pNK cells

(CD56<sup>+</sup>CD122<sup>+</sup>CD34<sup>+</sup> in human, CD122<sup>+</sup> NK1.1<sup>+</sup> DX5<sup>-</sup> in mouse) are subsequently cultured in the presence of IL-15 or IL-2 alone, the cells are able to develop into immature NK cells (CD122<sup>+</sup>CD16<sup>lo</sup> CD56<sup>+</sup>KIR<sup>-</sup> in human, CD122<sup>+</sup>CD2<sup>+</sup>NK1.1<sup>+</sup>DX5<sup>-</sup> Ly49<sup>-</sup> in mouse). Prolonged culture of pNK cells with IL-15 alone can generate the pseudomature lytic NK cells (CD122<sup>+</sup>CD2<sup>+</sup>NK1.1<sup>+</sup>DX5<sup>+</sup>CD94/NKG2<sup>+</sup> Ly49<sup>-</sup>), which have partial cytolytic activities. Several works have been shown that stromal cells provide both various cytokines and direct contact *via* cell surface receptors to developing NK cells, indicating that they are essential for the generation of lytic Ly49<sup>+</sup> mature NK cells as well as the maturation of NK cells (15,16). Thus, high frequency of activated lytic Ly49<sup>+</sup> mature NK cells (CD56<sup>+</sup>KIR<sup>+</sup>CD3<sup>-</sup> in human, CD122<sup>+</sup>CD2<sup>+</sup>NK1.1<sup>+</sup>DX5<sup>+</sup>CD94/NKG2<sup>+</sup> Ly49<sup>+</sup> CD3<sup>-</sup> in mouse) arises from the co-culture of pNK cells with stromal cells in the presence of IL-5 (13). At differential stages of NK cells during NK cell development, CD94, NKG2A, NKG2C and Ly49B were expressed at the early stages of development, and Ly 49G, Ly49C, Ly49I, orderly, and finally, Ly49A, D, E and F (17). After acquisition of these receptors, the developing NK cells acquire expression of several integrins in the order of  $\alpha_c$ , DX5, and Mac-1 during the maturation process followed by intensive expansion of NK cells.

Interaction between NK cell progenitors and stromal cells is required for the development of NK cells as shown in the cases of lymphotoxin (LT)-deficient mice and interferon regulatory factor (IRF)-1-deficient mice (15,18,19). The number of NK cells is profoundly reduced in the spleen and BM from LT $\alpha$ <sup>-/-</sup> mice, and impaired NK cell effector functions (15). Administration of LT $\beta$ R-Ig gives rise to a reduction of NK cell numbers, but adoptive transfer of wild type BM cells to LT $\alpha$ <sup>-/-</sup> mice did not restore splenic NK cells, suggesting that LT-dependent microenvironment is essential for NK cell development. However, treatments of IL-15 to BM cells from LT<sup>-/-</sup> mice restore NK cell population. It indicates that LT delivers a unique signal for NK cell development, which is independent of IL-15. NK cell development is also found to be impaired in IL-2R $\beta$ -deficient mice, characterized by a reduction in NK1.1<sup>+</sup>CD3<sup>-</sup> cells in the peripheral circulation and an absence of NK cytotoxic activity *in vitro* (16,20).

In addition to cell surface receptors and cytokine signals, transcription factors are also indispensable for the development of NK cells. pNK cells express transcription factors such as PU.1, GATA3, Id2, and Ets-1. Deficiency of ikaros (21), PU.1 (22), and Id2 (23) causes reduced numbers of pNK cells in mice. In HSCs, transcription factors such as myeloblastosis

(myb) oncogene, forkhead-related transcription factor 1C, c-myc, and Oct 2b may play important roles in the regulation of development or proliferation of pNK cells. c-myc has been reported as a key molecule for the commitment of murine erythroleukemia cell development (24), and c-myb regulates terminal development of HSCs through the regulation of hematopoietic commitment and progenitor cell proliferation (25). In pNK cells, immune regulators such as Fc $\gamma$  receptor, TNF receptor, IL-7 receptor, chemokine receptor, and CD36 seems to have critical roles in this stage. In mature NK cells, signaling molecules such as regulator of G protein signaling- $\gamma$  (RGS- $\gamma$ ), lymphocyte-specific protein tyrosine kinase, Fyn proto-oncogene, and mitogen activated protein kinase 1 may deliver positive or negative signals for NK cell maturation. It has been reported that overexpression of Id2 in HSCs markedly enhanced NK development, whereas the generation of other immune cells was blocked (23). Expression of Id2 is known to be correlated with p-NK activity. NK cells from fetal thymus of Id2<sup>-/-</sup> mice were markedly reduced, and pNK cells are completely missing, suggesting that Id2 is indispensable in thymic NK cell development, where it allows most probably restricts bipotent T/NK progenitors to the NK cell lineage. IRF-1 is also known to be necessary for the supporting NK cell development in the microenvironment, and IRF-1<sup>-/-</sup> mice have severe NK cell deficiency (19). When BM cells from IRF-1<sup>-/-</sup> mice are cultured in the presence of IL-15 during the lineage commitment of NK cells, and IL-15 gene expression is regulated by IRF-1. Adoptive transfer of BM cells of IRF-1<sup>-/-</sup> mice to wild type mice resulted in the recovery of functional NK cells. Ikaros<sup>-/-</sup> mice exhibit low levels of flt3 and c-kit (CD122) receptors, indicating that ikaros is also essential for pNK cell maturation (26). Also, Ets-1<sup>-/-</sup> mice showed the reduced number of splenic NK cells and the defective cytolytic activity against NK cell targets *in vivo* and *in vitro* (27). Ets<sup>-/-</sup> NK cells have normal levels of IL-2R- $\alpha$  and - $\gamma$ . Addition of cytokines such as IL-2, IL-15, IL-12, and IL-18 did not restore cytolytic activity of NK cells from Ets<sup>-/-</sup> mice. Mice deficient in PU.1, which is another member of the Ets family of transcription factors and regulates the expression of c-kit and IL-7 receptors, can generate functional NK cells from hematopoietic progenitors of PU.1<sup>-/-</sup> fetal liver cells, but they show reduced pNK cell numbers in BM and spleen (22,28). These mice fail to proliferate in response to IL-2 and IL-12, and lack Ly49 antigens. Clonal Ly49A acquisition is regulated by T cell factor-1 (TCF-1) during NK cell development, and TCF-1<sup>-/-</sup> mice show a selective reduction in Ly-49A expression (29,30). Mice that lack MEF, a member of the ETS family of

winged helix-turn-helix transcription factors, have a profound reduction in the number of NK and NKT cells (31). MEF<sup>-/-</sup> NK cells are defective in cytotoxicity against tumor cell targets, and produce minimal amounts of both IFN- $\gamma$  and perforin.

Recently, we have been reported that vitamin D<sub>3</sub> up-regulated protein 1 (VDUP1) is a stress-response gene which is up-regulated by 1,25 (OH)<sub>2</sub>D<sub>3</sub> in tumor cells. VDUP1<sup>-/-</sup> mice showed a profound reduction in the numbers of NK cells, and in NK cell activity. In the VDUP1<sup>-/-</sup> mice, the expression of CD122 was reduced. Our data indicated that VDUP1 is also required for CD122 expression and NK cell maturation (32).

### NK cell receptors and their ligands

NK cell effector functions are regulated by a balance between activating receptors and inhibitory receptors that interact with their ligands such as MHC class I or MHC class I-related molecules (non-classical MHC class I) on the target cells (33)(Table 1). These receptors are divided into two structural families: immunoglobulin superfamily (leukocyte inhibitory receptors, killer cell Ig-like receptors (KIR, CD158) and C-type lectin-like family (NKG2D, CD94/NKG2, lymphocyte antigen 49 (LY49)).

NK cell activating receptors are essential for the activation of NK cell effector functions, which results in either cytotoxicity and/or cytokine production. Natural cytotoxicity receptors (NCRs) consist of the receptor NKp30, NKp44, and NKp46, and all of which play a key role in NK-cell-mediated lysis of tumor cells upon signaling through the differential association with the adaptor molecules (DAP12, Fc $\gamma$ , or CD3 $\zeta$ ) (34,35). Indeed, mice that lack the cell surface adaptor molecules have defect in NK-mediated tumor cell killing (35). In contrast to other NCRs, the expression of NK p44 (DAP12/KARAP) is restricted only in IL-2-activated NK cells (36). MICA (MHC class I chain-related molecule) is known as a stress-inducible human nonclassical MHC class I molecule, and their receptor, NKG2D, is predominantly expressed on NK cells,  $\gamma\delta^+$  T cells, CD8<sup>+</sup>  $\alpha\beta$  T cells (37). Activating signals are triggered by association of NKG2D homodimers with DAP10 or DAP12 transmembrane signaling adaptor molecule, which has an immunoreceptor tyrosine-based activating motif (ITAM) domain (38)(Fig. 2). Following receptor-ligand association, ITAM-containing adaptor molecules recruit src family kinases at the tyrosine residues (35). NKG2D/DAP10 receptor complex transmits activating signals through recruiting the p85 subunit of the phosphatidylinositol-3-kinase (PI3K)(39), leading to NK cell-mediated cytotoxicity against MICA-bearing tumors (39,40), whereas DAP12 can trigger Syk family protein tyrosine

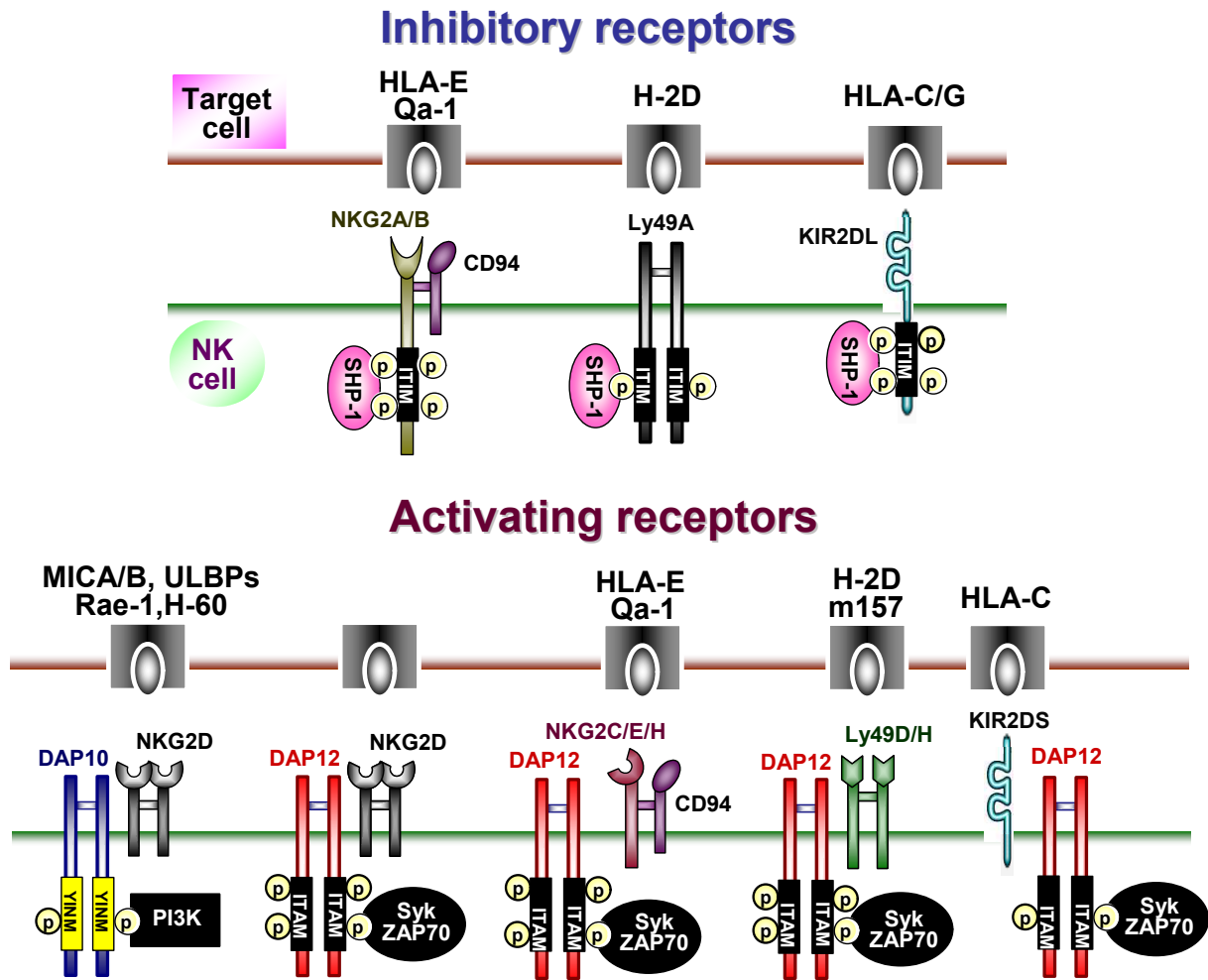
**Table I.** Activating and inhibitory receptors of NK cells

Receptor	Ligands	Function	Species
NKG2A	HLA-E/Q <sup>a</sup> -1 <sup>b</sup>	Inhibition	H, M
NKG2C	HLA-E/Q <sup>a</sup> -1 <sup>b</sup>	Activation	H, M
NKG2D	MICA/B, ULBPs (Human), Rac-1, H60 (mouse)	Activation	H, M
NKG2E	HLA-E/Qa-1b	Activation	H, M
NKp30 (NCR3)	Unknown	Activation	H
NKp44 (NCR2)	Unknown	Activation	H
NKp46 (NCR1)	Unknown	Activation	H, M
NKR-P1A	Unknown	Activation	H, M
NKR-P1B	Unknown	Inhibition	M
NKR-P1C	Unknown	Activation	M
NKR-P1D	Unknown	Inhibition	M
NKR-P1F	Unknown	Activation	M
Ly49A	H-2D	Inhibition	M
Ly49C	H-2D, H-2K	Activation	M
Ly49D	H-2D	Activation	M
Ly49E	Unknown	Inhibition	M
Ly49G	H-2D	Inhibition	M
Ly49H	MCMV m157	Activation	M
KIR2DL	HLA-C/G	Inhibition	H
KIR2DS	HLA-C	Activation	H
KIR3DL (CD158)	HLA-A, HLA-Bw4	Inhibition	H
CD2	CD58 (LFA-3 <sup>†</sup> )	Activation	H, M
CD16	IgG	Activation	H, M
CD28	B7.1/B7.2	Activation	H, M
CD40L	CD40	Activation	H, M
CD226	CD112 (Nectin-2), (DNAM-1 <sup>‡</sup> ) CD155 (PVR*)	Activation	H
CD244 (2B4)	CD48	Inhibition	H, M
ILT2	HLA-G	Inhibition	H
ILT4	HLA-F	Inhibition	H
P75/AIRM1	Sialylated sugars moieties	Inhibition	H

<sup>†</sup> LFA-3, lymphocyte function-associated antigen; <sup>‡</sup> DNAM, DNAX accessory molecule; \*PVR, Polio virus receptor.

kinases. An association of Ly49H NK cell activating receptor with DAP12 triggers NK cell-mediated cytotoxicity and cytokine expression (41).

UL16-binding proteins (ULBPs), another NKG2D ligands, that are expressed on stressed, infected, or tumor cells bind to the human cytomegalovirus glycoprotein UL16 (42), and their engagements by NKG2D allow NK cells to kill target cells. It has been known that NK cell inhibitory receptors bind to the MHC class I ligands more strongly than the do activating receptors, suggesting that the balance between inhibitory and activating receptor signaling in favor of activation (43-45). Binding of NK cell inhibitory receptors with their ligands renders the target cells to be protected from NK cell-mediated cytotoxicity. Signaling *via* NK cell inhibitory receptors is usually



**Figure 2.** Interaction between NK cell receptors and their ligands. NK cell function is regulated by activating and inhibitory signaling. When NK cell inhibitory receptors bind their ligands (eg, HLA-E in human, and Qa-1 in mouse; classical or non-classical MHC class I molecules) on the surface of the target cells, ITIM domains in the cytoplasmic tails of the inhibitory receptors (NKG2 isoforms such as NKG2A and NKG2B heterodimerized with CD94 lectin-like receptor, Ly49A, and KIR2DL) on NK cells are phosphorylated at tyrosine residues. This phosphorylation induces the recruitment of Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1), leading to inhibitory signaling via the dephosphorylation of signaling molecules. The long isoform of NKG2D (NKG2DL) is predominantly expressed in resting NK cells, and associated with DNAX-activating protein of 10kDa (DAP10), while the expression of short isoform (NKG2DS) is upregulated in activated NK cells. Upon ligation, YNIM motifs of the DAP10 adaptor protein are phosphorylated, and the activation of phosphatidylinositol-3 kinase (PI3K) is induced. In contrast to NKG2D/DAP10 complexes, the phosphorylation is occurred in ITAM domains of DAP12 associated with the activating receptors (NKG2D, CD94-NKG2 isoforms, Ly49 isoforms, and KIR2DS) followed by the recruitment and activation of Syk or ZAP-70 protein tyrosine kinases capable of activating NK cells.

triggered by immunoreceptor tyrosine-based inhibitory motif (ITIM) domains in the cytoplasmic tails of these NK receptors. The phosphorylation of ITIM domain by ligand binding is capable of recruitment of src homology containing protein tyrosine phosphatases (SHPs) followed by blocking of NK effector functions (43,46). Studies in gene-disrupted mice have shown that disrupting interactions of KIR with their ligands on tumor cells *in vivo* may enhance antitumor responses mediated by both innate and adaptive immune effector cells (47). In addition, CD94-NKG2A com-

plex inhibits CD16-mediated NK cell cytotoxicity through ADCC in tumour cell lines (48).

NK cells that also express a number of co-stimulatory receptors including CD27, CD28/ICOS (inducible co-stimulator) and CD154 stimulate NK cell activation (37,49). The constitutive expression of CD27, a member of the TNF receptor superfamily, is up-regulated on IL-2-activated NK cells. CD27-mediated activation appears to play an important role in the NK cell-mediated innate immunity against virus-infected or transformed cells expressing its ligand (CD70)(50,49).

It has been reported that CD70 expression promoted the rejection of MHC class I-deficient tumor, which is mediated by NK cells and granule exocytosis-dependent mechanisms (8). Since the NK cell-mediated tumor rejection gives rise to the subsequent tumor-specific cytotoxic and T helper 1 (Th1) responses, these data raise the possibility that CD27-mediated activation by CD70 may be a conclusive evidence for the relation between the innate immunity and adaptive immunity. The murine NK cell-mediated cytotoxicity against tumor cells is dependent on interaction between CD28 and their ligands, CD80 (B7.1), but this interaction is not involved in triggering of human adult NK cells (51-53).

In addition to interactions between CD40L (CD154) and CD40, murine CD40 and CD86 (B7.2) trigger murine NK cell-mediated cytotoxicity against tumor cells, suggesting that they have ability to interact with receptors on NK cells other than CD40L and CD28, respectively (54,55). Since engagement of 2B4 (CD244) on NK-cell surfaces with specific antibodies or its ligand CD48 is capable of triggering lysis of target cells and interferon- $\gamma$  secretion, 2B4 is originally known as an activating receptor, so that it may contribute to the NK cell effector functions. However, this is shown to function as an inhibitory receptor at early stages of NK cell development to prevent killing of normal autologous cells, rather than as an activating receptor (56). As a consequence, it is likely that its role is depend upon the interaction with NCRs, and remains inconclusive.

### **NK cell effector functions by cytokine regulation**

Immunoregulatory cytokines including IFN- $\alpha$ , IFN- $\beta$ , IL-2, IL-12, and IL-15 are able to stimulate the effector functions of resting NK cells, which represent activity for cytotoxicity, survival, and proliferation as well as innate immunity against virally infected cells and tumor cells (37,57,58). It is believed that IL-2, IL-12 and IL-15 may be useful for tumor immunotherapy (13,14). Long-term immunotherapy with low-dose IL-2 and IFN- $\alpha$  in patients with advanced renal cell carcinoma resulted in clinical response rates and survival probabilities that are similar to those obtained using higher doses of IL-2 (37). In human, CD56<sup>bright</sup> NK cell subset also produces several cytokines including IFN- $\gamma$ , TNF- $\alpha$ , TNF- $\beta$ , IL-10 and GM-CSF (61), but CD56<sup>dim</sup> NK cell subset do not produce these cytokines (2,4). Although immature NK cells can produce Th2 cytokines such as IL-5 and IL-13, the ability to produce Th2 cytokines is lost upon terminal development; instead, mature NK cells acquire ability to produce IFN- $\gamma$  (62). IFN- $\gamma$  has been demonstrated to stimulate adap-

tive immunity, antigen presentation by upregulating MHC class I and II molecules, and inhibit tumor angiogenesis (63-65). This angiogenesis *in vivo* can be suppressed by IFN- $\gamma$ , which is produced. by administration of IL-12 (63). It has been shown that TNF- $\alpha$  effectively stimulates not only IFN- $\gamma$  production by human NK cells when combined with IL-1 $\beta$  or IL-12 (66) but also tumor rejection due to the recruitment of NK cells into tumor or inflammatory sites (67,68). Although IL-10 did not induce the production of IFN- $\gamma$  by NK cells, but it enhanced the ability of IL-18 to stimulate NK cell production of IFN- $\gamma$  (69). Both IL-12 and IL-18 have been demonstrated to act synergistically to enhance *in vitro* antitumor capacity and IFN- $\gamma$  production mediated *via* NK cells (70-73). Several studies have been reported that the antitumor effect that mediated by locally secreted IL-12 is augmented by systemic treatment with IL-18 (74), and that systemic administration of IL-18 alone results in not only suppression of tumor growth, but also increased survival rates of tumor-bearing mice (26,75-78). Furthermore, co-treatment of IL-18 with IL-10 stimulated NK cell proliferation, cytotoxicity, and IFN- $\gamma$  production (69, 79,80). Flt 3L (fms-like tyrosine kinase 3 ligand) is also known to induce antitumor immune responses *in vivo* (81), and the expansion of DC, which promoted NK cell-dependent anti-tumor effects in mice with MHC class I-negative tumors (82). IFN- $\gamma$  production and cytotoxic activity through activated murine NK cells are promoted by IL-21 and GM-CSF, but did not support their viability (83). Moreover, NK cell responses *via* IL-15-induced expansion of resting NK cells are restricted by IL-21, but promoted antigen-specific T cell activation. Thus, IL-21 prevented the initiation of further innate responses, indicating that it may be able to promote the transition from innate to adaptive immunity.

Meanwhile, NK cells secrete and respond to a number of chemokines including XCL1, CCL1, CCL3, CCL4, CCL5, CCL22, and CXCL8 (84), which are regulated, in part, by IL-15 (85-87), or IL-2 (88). These chemokines play an important role in NK cell homing to infected and neoplastic cells in secondary lymphoid tissues, where their production of IFN- $\gamma$  may serve to directly regulate T cell responses (84). While resting CD56<sup>dim</sup>/CD16<sup>+</sup> NK cell subsets express CXCR1, CXCR2, CXCR3, and CXCR4, CD56<sup>bright</sup>/CD16-NK cells express high levels of CCR5 and CCR7. Cytolytic activity of NK cells is stimulated by CCL2, CCL3, CCL4, CCL5, CCL10, and CXCL1.

### **NK cell cytotoxicity by granule exocytosis**

Among NK cell-mediated target cell killing mechanisms, the release of cytoplasmic granules such as

perforin and granzymes (granule exocytosis) corresponds to the principal function (89). Most granule proteins are predominantly expressed in cytotoxic lymphocytes, including NK cells, CTLs, NKT cells and  $\gamma\delta^+$  T cells. Perforin, a membrane-disrupting protein forms transmembrane pores in target cell membranes, and granzymes, a family of serine proteases, trigger apoptotic cell death either directly through caspase activation, or through caspase-independent pathways. Perforin-mediated killing appears to be promoted by IL-12-activated NK cells and mainly occurred in the lung and spleen, compared with the liver (90-92). NK activity and perforin expression were reduced in human immunodeficiency virus (HIV)-infected patients (93) and perforin expression was restored by *in vivo* administration of IFN- $\alpha$ . Both IL-12 and IL-21 induced the activation of NK cells, and suppression of various tumors in a perforin-dependent manner (72,94).

In the presence of perforin, synergistic effects of granzyme A and granzyme B on target cell death has been implicated to be triggered by nuclear damage (95). Recently, granzyme A and granzyme M that have been determined to induce caspase-independent cell death, and generate single stranded DNA nicks (96). In another molecule regarding granule exocytosis, granulysin known as a member of the saposin-like protein family including amoebapores and NK lysin has lytic activity against both microbes and tumors, but its precise mechanism in NK cell-mediated cytotoxicity remain to be further investigated (97-99).

### NK cell cytotoxicity by death receptors

Expression of death receptors on target cell surface results in apoptosis signal *via* binding of a specific death ligand on NK cells in a caspase-dependent manner (100). The major family of death receptors is represented by TNF receptors (R), which consist of Fas (CD95, Apo-1), TNFR1 (p55, CD120a), TNF-R2 (p75, CD120b), death receptor 3 (DR3), death receptor 4 (DR4, TNF-related apoptosis inducing ligand (TRAIL)-R1) and death receptor 5 (DR5, TRAIL-R2). Since NK cells express members of TNF family ligands, NK cell-mediated apoptosis is dependent on the expression of caspases (101). RMA-S T lymphoma cells that do not normally express Fas, but it is upregulated directly by NK cells, and thereafter NK cells kill them in a Fas-dependent manner, indicating that death receptor-mediated apoptosis has a more prominent role in the clearance of NK-sensitive tumors (102). In particular, FasL expression on NK cells is shown to be involved in suppression of tumor metastases by IL-18 (103,104). Although activated NK cells expressing TRAIL that induced by IL-2, IFNs, or IL-15 represent a potent anti-tumorigenic effect (105), tumor initiation and meta-

stasis is augmented in TRAIL-deficient mice (106). Furthermore, TRAIL expression on NK cells is shown to eliminate immature DC *in vivo* and limit DC vaccination efficacy (107,108).

### Concluding Remarks

In addition to the relation of NK cells with virus infection or tumor, NK cells are known to be involved in the pathogenesis of diseases such as asthma, graft-versus-host disease, Hodgkin's disease, and systemic lupus erythematosus, while they can selectively suppress the induction of some autoimmune diseases.

Despite the emerging role of NK cells as a powerful tool for tumor immunotherapy have been deciphering as a key linker between innate and adaptive immunity, the detailed mechanisms regarding the development or biological function of the different NK subsets is still unclear. Antigens derived from NK cell-mediated cytotoxicity against their targets stimulate DC maturation and activation, resulting in the enforcement of innate immunity followed by adaptive immunity through the triggering of subsequent T cell responses. IFN- $\gamma$ -producing activated NK cells is also involved in the induction of antigen presentation by upregulating MHC class I and II molecules. As mentioned above, recent evidences indicate that the cross-talk of NK cells with DC plays a pivotal role in the triggering of adaptive immunity through the priming and/or stimulation of adaptive immune cells.

Tumor escape from NK cell attack might be derived from the dysregulation of adhesion molecules, co-stimulatory molecules, ligands for ITAM-bearing NK-cell receptors, and inflammatory/immunosuppressive cytokines on the NK cell or target cell. In NK cell-based tumor immunotherapy, the regulation of balance between inhibitory and activating receptor signaling and the discovery of the good candidates to augment both differentiation and effector function will be a powerful approach of clinical trials for tumor therapy. In addition, introducing immune responses inside tumor tissues is also capable of improvement of the NK-stimulating microenvironment.

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